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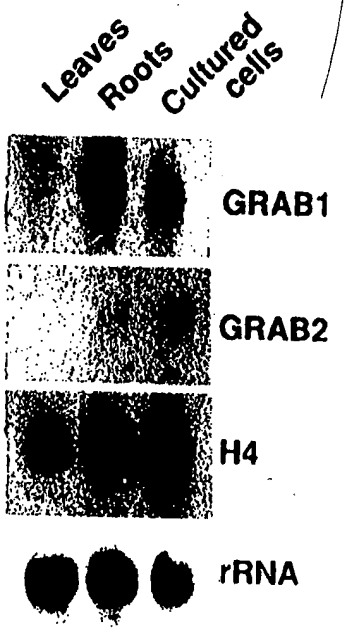
(57) Abstract

A method of controlling plant cell and plant virus growth and/or replication, plant cell cycle, differentiation, development and/or scenescence is provided characterised in that it comprises increasing or decreasing the levels or binding capabilities of GRAB (Geminivirus RepA Binding) proteins other than Rb (Retinoblastoma) proteins within plant cells.



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PLANT GRAB PROTEINS.

The present invention relates to methods of controlling plant cell cycle, particularly for the purpose of controlling plant cell and plant virus growth and/or replication, differentiation, development and/or scenescence; to use of previously unidentified and/or unisolated proteins and/or nucleic acids in such methods; to use of known proteins and nucleic acids of previously unknown native function in such methods; to the unidentified and/or unisolated proteins and nucleic acids *per se* and in enriched, isolated, cell free and/or recombinant form; and to plants comprising such recombinant nucleic acids.

It has been well documented that successful completion of viral replication cycles within the infected cell usually requires the participation of cellular factors. This is particularly evident in the case of viruses with small genomes that encode just a few proteins. For example, animal DNA tumor viruses use the cellular machinery for their transcriptional and DNA replication processes. In addition one or more virally-encoded proteins have evolved that directly impinge on the infected cell physiology to create a cellular environment appropriate for viral replication. One typical example is that of the oncoproteins encoded by animal DNA tumor viruses, i. e., SV40 T antigen, adenovirus E1A or human papilloma virus E7 proteins, which activate cell cycle in the infected cell by interfering with the retinoblastoma pathway (26, 28, 45).

A similar strategy seems to have evolved in plant geminiviruses, a unique group of plant DNA viruses. The geminivirus genome consists of 1 or 2 small (2.6-3.0 kb) circular single-stranded DNA molecules, depending on the subgroups (11, 24). Wheat dwarf geminivirus (WDV) belongs to subgroup I whose members have the smallest genome, a single ssDNA molecule, 2750 nucleotides in length, which encodes only a few proteins. Among them, RepA (also called CI) and Rep (also called CI:C2) are the only WDV proteins required for viral transcription and replication (24). RepA is translated from the single transcript produced under the control of the complementary-sense promoter. After a splicing event of this mRNA, the Rep protein is produced (37). WDV Rep, absolutely required for viral DNA replication and this is homologous to the Rep proteins of all geminiviruses. Geminivirus Rep has been shown to have DNA nicking-joining activity in vitro, origin-recognition ability and ATPase activity. However, RepA protein is unique to the WDV geminivirus subgroup and has been

implicated in modulation of Rep activity, binding to plant retinoblastoma (Rb) protein (45, 46) and stimulation of virion-sense gene expression. In addition, we have recently shown that in WDV, the Rb-binding protein (RepA) and the initiator protein (Rep) seem to play coordinate roles during viral DNA replication.

Geminivirus DNA replication occurs in the nucleus of the infected cells and, due to the lack of replicative enzymes encoded by the viral genome, it requires S-phase functions. Consistent with this is the accumulation of replicative intermediates in S-phase nuclei (1). Geminiviruses normally infect non-proliferating cells but, interestingly, they induce the appearance of cellular proteins typical of S-phase, such as proliferating cell nuclear antigen (PCNA) (29) which is otherwise undetectable in non-proliferating cells. Subgroup I geminiviruses such as WDV encode proteins containing a LXCXE motif in the RepA protein, which mediates its ability to interact with Rb, involved in the mechanism by which geminiviruses impinge on the cell cycle activation circuit (45). These observations served the basis to isolate a full-length cDNA encoding ZmRbl, a plant Rb protein, which could act in plant cells as a regulator of the Gl/S transit (46). Consistent with this function, overexpression of plant Rb (as well as human Rb) in cultured plant cells significantly inhibits WDV DNA replication (45, 46). Therefore, it seems that at least one of the mechanisms used by geminiviruses to favour DNA replication is the triggering of an S-phase in the infected cell by sequestering Rb and, consequently, by interfering with its negative cell growth activity.

Regulation of cell cycle, growth and differentiation in plants is the result of a complex interplay of regulators whose activity is the response to a wide variety of signals such as hormones, nutrient availability or environmental conditions (20, 39). For example, a rapid increase in the levels of D-type cyclin mRNAs occurs in response to sucrose or cytokinin treatment (41) while those of the cyclin-dependent kinase (cdc2) mRNAs depends on the presence of auxin. The molecular nature of other plant cell cycle regulators as well as their function in connection to cell growth and differentiation remains largely unknown. Therefore, it is important to identify the cellular factors involved in these control pathways to elucidate the molecular mechanisms governing the response of plant cells to growth signals.

Due to the absolute requirement for cellular factors to complete geminivirus replication, the present inventors postulated that geminiviruses might modulate cell

physiology by mechanisms other that the interference with the Rb pathway and that such effect might be the consequence of the targeting of, so far, unknown cellular factors by the geminivirus proteins. They have used an experimental strategy to identify proteins that interact functionally with RepA, the Rb-binding protein of WDV, and now have provided several cDNA clones encoding previously unidentified proteins and determined their function.

Based on amino acid sequence analysis, these proteins have been determined to share a common N-terminal domain, required for interaction with the viral RepA protein, while their C-terminal domains are unique to each of them. They may represent members, likely with transcriptional regulatory activity, of a much larger family of proteins related to regulators of hormone and nutrient response, meristem development and plant senescence.

Thus in a first aspect of the present invention there is provided a method of controlling plant cell cycle characterised in that it comprises increasing or decreasing the levels of GRAB (Geminivirus RepA Binding) proteins or peptides or increasing or decreasing the binding capabilities of GRAB proteins or peptides. within plant cells. Such control, *inter alia*, allows control of plant cell growth and/or replication, plant virus growth within cells, plant cell differentiation, development and/or scenescence. It will be understood that such proteins and peptides are other than Rb (Retinoblastoma) proteins, being particularly those described herein below with regard to the sequence listing and their functional variants.

Increasing or decreasing the levels of GRAB proteins peptides may be achieved by overproducing or underproducing the protein or peptide in a plant cell, that is, as compared to the normal level of production of the protein or peptide in the cell Decrease of native GRAB binding activity may be achieved eg. by application of a GRAB proetin or peptide binding agent, eg. such as WDV RepA or a functional part or variant thereof.

Particularly the GRAB proteins or peptides for use in this method are those comprising an amino acid sequence SEQ ID No 2 or 4 as shown herein or a functional variant thereof that is capable of binding Geminivirus RepA. Preferred proteins or peptides have amino acid sequence homology of at least 70% with that of SEQ ID No 2 or 4, more preferably at least 90% and most preferably at least 95%. Particularly the

GRAB proteins are those in which the first 200 N-terminal amino acids are capable of binding to viral RepA protein; more preferably the first 170 N-terminal. amino acids are so capable and most preferably the first 150 amino acids.

These methods may comprise the direct application of such GRAB proteins or peptides to plant cells or whole plants, but more conveniently will comprise use of the corresponding GRAB protein or peptide encoding or antisense nucleotides, ie.nucleic acids placed within the cells, particularly by use of recombinant nucleic acid, eg. recombinant DNA comprising a GRAB protein or peptide encoding sequence, positioned in the cell behind a promotor capable of supporting GRAB protein or peptide expression or production of antisense RNA. GRAB protein encoding nucleic acids can be used to produce GRAB where required, eg. ectopically in a tissue where it is not normally expressed, eg. vegetative tissue or stem tissue such as xylem or phloem. An alternative strategy might comprise expressing a GRAB protein binding peptide, eg. Geminivirus RepA, a functional variant thereof or a GRAB protein binding portion thereof, such as the C-terminal portion. Such a peptide would bind to native GRAB proteins and inhibit their activity. It will be realised that any expression of RepA, and particularly only a GRAB protein binding part thereof such as a RepA with a truncated N-terminal, in a transgenic plant other than that produce by a whole intact genimivirus would be novel. A RepA encoding cDNA in functional relationship with a promoter or other regulatory sequence in a DNA or RNA vector or DNA construct would be particularly useful for such purpose.

It will be realised that a most effective method of delivering proteins and peptides of the invention to plant cells is by expressing nucleic acid encoding *them in situ*. Such method is conventionally carried out by incorporating oligonucleotides or polynucleotides,having sequences encoding the peptide or protein, into the plant cell DNA. Such nucleotides can also be used to downregulate native GRAB expression by gene silencing coexpression (6) or through antisense strategy. By use of mutagenesis techniques, eg. such as SDM, the nucleotides of the invention may be designed and produced to encode proteins and peptides which are functional variants or otherwise overactivated or inactivated, eg. with respect to binding, of the invention

It will be realised by those skilled in the art that suitable promotors may be active continuously or may be inducible. It will be appreciated by those skilled in the art

that inducible promotors will have advantage in so far as they are capable of providing alteration of the aforesaid GRAB protein activity only when required, eg. when viral infection is threatened, or when the plant would otherwise be particularly vulnerable, or at a particular stage of cell development. Such promoters may for example be induced by environmental conditions such as stress inducing conditions, eg. reduced water availability caused by drought or freezing, or by complex entities such as plant hormones, eg. plant to plant signalling stress hormones, or by simpler entities such as particular cations or anions eg. metal cations. No particular limitation on the type of promoter to be used is envisoned.

Numerous specific examples of methods used to produce transgenic plants by the insertion of cDNA in conjunction with suitable regulatory sequences will be known to those skilled in the art. For example, plant transformation vectors have been described by Denecke et al (1992) EMBO J. 11, 2345-2355 and their further use to produce transgenic plants producing trehalose described in US Patent Application Serial No. 08/290,301. EP 0339009 B1 and US 5250515 describe strategies for inserting heterologous genes into plants (see columns 8 to 26 of US 5250515). Electroporation of pollen to produce both transgenic monocotyledonous and dicotyledonous plants is described in US 5629183, US 7530485 and US 7350356. Further details may be found in reference works such as Recombinant Gene Expression Protocols. (1997) Edit Rocky S. Tuan. Humana Press. ISBN 0-89603-333-3; 0-89603-480-1. It will be realised that no particular limitation on the type of transgenic plant to be provided is envisaged; all classes of plant, monocot or dicot, may be produced in transgenic form incorporating the nucleic acid of the invention such that GRAB activity in the plant is altered, constituitively, ectopically or temporally.

A preferred embodiment of the first aspect of the invention provides a method of producing or inhibiting senescence in a plant cell comprising increasing or decreasing the levels or activity of a GRAB protein or peptide, particularly a GRAB1 protein of SEQ ID No 10 or a functional variant therof capable of inducing senescence in *N.bentamiana* plants, in a plant cell. Again such increase or decrease is most effectively achieved through incorporation of nucleic acid, in this case of SEQ ID No 9, or a functional variant thereof, or may be achieved by use of RepA encoding DNA .

A second aspect of the present invention provides novel GRAB proteins or peptides *per se* and in enriched, isolated, cell free and/or recombinantly produced form. Such proteins or peptides may be naturally occurring or may be conservatively substituted homologues thereof as referred to below. Preferred proteins and peptides have an N-terminal sequence having 90% or more homology to the N-terminal 200 (more preferably to the first 170 and most preferably the first 150) amino acids of GRAB1 or GRAB2 described herein, more preferably 95% or more and most preferably 98% or more. Preferred peptides comprise the sequence of the first 150 to 200 amino acids of either of these sequences or conservatively substituted variants thereof. Preferred peptides comprise such a sequence without the C-terminal sequence of SENU, NAM, ATAF1 or ATAF 2 shown in Figure 4 attached hereto.

Particularly the GRAB proteins and peptides are those comprising an amino acid sequence SEQ ID No 3 or 4 as shown herein or a functional variant thereof that is capable of binding Geminivirus RepA and have amino acid sequence homology of at least 70% with that of SEQ ID No 3 or 4, more preferably at least 90% and most preferably at least 98%. More preferably they comprise SEQ ID No 6 or 8 or such homology limited functional variant thereof and most preferably SEQ ID No 10 or 12 or such homology limited functional variant thereof. Where the protein or peptide comprises SEQ ID No 3 or 4 it is not of SENU, NAM, ATAF1 or ATAF2.

Proteins or peptides may be derived from native protein or peptide encoding DNA that has been altered by mutagenic techniques eg. using chemical mutatgenesis or mutagenic PCR.

A third aspect of the present invention provides GRAB protein or peptide encoding and antisense nucleic acid *per se* and in enriched, isolated, cell free and/or recombinant form. Particularly provided is consense and antisense DNA in the form of individual oligonucleotides and polynucleotides, provided that said DNA does not encode the full amino acid sequence of SENU, NAM, ATAF1 or ATAF2 as shown in Figure 4.

Specifically provided is nucleic acid, eg. in the form of a nucleotides, but preferably in the form of recombinant DNA or cRNA (mRNA), that codes for the expression of the GRAB protein having an N-terminal sequence with at least 60% homology with the first 200 N-terminal amino acids of GRAB1 or GRAB2 as described

herein ; ie. its first 200 codons having such homology. Preferably the homology is at least 75% and most preferably at least 90%.

Preferred nucleic acid is DNA or RNA comprising of SEQ ID No 1, 2, 5, 7, 9 or 11 or a functional variant thereof having the homology limtations referred to above. More preferred is DNA of SEQ ID No 9 or 11 or a functional variant thereof.

With respect to the present specification and claims, the following technical terms are used in accordance with the definitions below unless otherwise specified.

A "functional variant" of a peptide, protein, nucleotide or polynucleotide is a peptide, protein, nucleotide or polynucleotide the amino acid or base sequence of which can be derived from the amino acid or base sequence of the original peptide, protein, nucleotide or polynucleotide by the substitution, deletion and/or addition of one or more amino acid residues or bases in a way that, in spite of the change in the amino acid or base sequence, the functional variant retains at least a part of at least one of the biological activities of the original peptide, protein, nucelotide or polynucleotide in that is detectable for a person skilled in the art. A functional variant is generally at least 50% homologous (i.e. the amino acid or base sequence of it is 50% identical), but advantageously at least 70% homologous and even more advantageously at least 90% homologous to the native or synthetic sequence from which it can be derived. Any functional part of a protein or a variant thereof is also termed functional variant.

The term "overproducing" is used herein in the most general sense possible. A special type of molecule (usually a protein, polypeptide or oligopeptide or an RNA) is said to be "overproduced" in a cell if it is produced at a level significantly and detectably higher (e.g. 20% higher) than natural level. Overproduction of a molecule in a cell can be achieved via both traditional mutation and selection techniques and genetic manipulation methods.

The term "ectopic expression" is used herein to designate a special realisation of overproduction in the sense that, for example, an ectopically expressed protein is produced at a spatial point of a plant where it is naturally not at all (or not detectably) expressed, that is, said protein or peptide is overproduced at said point.

The term 'underproducing' is intended to cover production of peptide, polypeptide, protein or mRNA at a level significantly lower than the natural level (eg. 20% or more lower), particularly to undetectable levels.

The DNA or RNA of the invention may have a sequence containing degenerate substitutions in the nucleotides of the codons in the sequences encoding for GRAB proteins or peptides, eg. GRAB1 or GRAB2 , and in which the RNA U's replace the T's of DNA. Preferred *per se* DNAs or RNAs are capable of hybridising with the polynucleotides encoding for GRAB1 or GRAB2 in conditions of low stringency, being preferably also capable of such hybridisation in conditions of high stringency.

The terms "conditions of low stringency" and "conditions of high stringency" are of course understood fully by those skilled in the art, but are conveniently exemplified in US 5202257, columns 9 and 10. Where modifications are made they should lead to the expression of a protein with different amino acids in the same class as the corresponding amino acids to these GRAB protein sequences; that is to say, they are conservative substitutions. Such substitutions are known to those skilled in the art (see, for example, US 5380712), and are considered only when the protein is active as a GRAB protein.

In a fourth aspect of the present invention there is provided a protein or peptide expressed by the recombinant DNA or RNA referred to in the second aspect above, new proteins or peptides derived from that DNA or RNA and protein or peptide that is produced from native DNA or RNA that has been altered by mutagenic means such as the use of mutagenic polymerase chain reaction primers. Methods of producing the proteins or peptides of the invention characterised in that they comprise use of the DNA or RNA of the invention to express them from cells are also provided in this aspect.

A fifth aspect of the present invention provides nucleic acid probes and primers complementary to any 15 or more contiguous bases of the DNA sequences identified herein below as SEQ ID No 5, 7, 9 or 11 or complemetary sequences or RNA sequences corresponding thereto; particularly of the first 150 N-terminal coding DNA bases of such sequences. These probes and primers in the form of oligonucleotides and polynucleotides may also be used to identify further naturally occuring or synthetically produced GRAB peptides or proteins using eg. southern or northern blotting'

Oligonucleotides for use as probes conveniently comprise at least 18 consecutive bases of the sequences SEQ ID No 5, 7, 9 or 11 herein, preferably being of 30 to 100 bases long, but may be of any length up to the complete sequence or even longer. For use as PCR or LCR primers the oligonucleotide preferably is of 10 to 20

bases long but may be longer. Primers should be single stranded but probes may be double stranded ie. including complementary sequences.

A sixth aspect of the present invention provides vectors comprising DNA or RNA of the third aspect of the invention.

A seventh aspect of the present invention provides a method for producing transformed cells comprising nucleic acid of the invention comprising introducing said nucleic acid into the cell in vector form.

A eighth aspect of the present invention provides a method for producing transformed cells comprising nucleic acid of the invention comprising introducing said nucleic acid into the cell directly, eg. by electroporation.or particle bombardment. Particularly provided is the electroporation of pollen cells.

An ninth aspect of the present invention provides cells, particularly plant cells, eg. including pollen and seed cells, comprising the recombinant nucleic acid of the invention, particularly the DNA or RNA of the invention, and plants comprising such cells.

Plasmids containing a DNA coding for expression of the GRAB proteins GRAB 1 and GRAB 2 described herein have been deposited under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms of 1977; these being deposited on 11 June 1997 at the Coleccion Espanola de Cultivos Tipo, with the accession numbers CECT 4889 (this containing GRAB 1 sequence) and CECT 4890 (this containing GRAB 2 sequence).

SEQUENCE LISTING

SEQ ID No 1 and 2 show the nucleotide sequences of GRAB1 and GRAB 2 respectively which encode for conserved domains N1 to N5 with intervening bases marked as N.

SEQ ID No 3 and 4 show the respective amino acid sequences corresponding to SEQ ID No 1 and 2.

SEQ ID No 5 and 7 show the full nucleotide sequences spanning N1 to N5 of GRAB1 and GRAB2 respectively.

SEQ ID No 6 and 8 show the corresponding amino acid sequences to SEQ ID No 5 and 7.

SEQ ID No 9 and 11 show the full length sequences of isolated cDNA including coding regions for GRAB1 and GRAB2 respectively.

SEQ ID No 10 and 12 show the corresponding amino acid sequences of proteins GRAB1 and GRAB2.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of northern analysis for transcripts of GRAB 1 and GRAB 2.

Figure 2 shows the results of studies carried out to to identify the region of GRAB 1 and GRAB 2 which are involved in the binding to WDV Rep A.

Figure 3 shows the results of studies carried out to identify the region of WDV Rep A involved in the binding with GRAB proteins.

Figure 5 shows the alignment of various protein sequences, previously known and unknown, having the GRAB protein domains N1 to N5, for use in the method of the invention.

Figure 6 shows the charge distribution of these proteins.

The present invention will now be described further by way of illustration only by reference to the following non-limiting Examples. Further embodiments falling within the scope of the claims will occur to those skilled in the art in the light of these.

In the Examples below the following methods were used.

MATERIALS AND METHODS

DNA manipulations

Proteinase K, restriction endonucleases and other enzymes for DNA manipulations were from Merck, Boehringer Mannheim, New England Biolabs and Promega. Standard DNA manipulation techniques were applied as described in [34]. DNA sequencing was carried using an Applied Biosystem automatic sequencing device. Oligonucleotides were from Isogen Bioscience BV (Maarsen, The Netherlands).

DNA and RNA purification

Genomic DNA and total RNA were isolated from wheat leaves, roots and suspension cultured cells by grinding the material, previously frozen in liquid nitrogen, essentially as described [41]. The powder was mixed with extraction buffer (50 mM Tris-HCl, pH 6.0, 10 mM EDTA, 2% SDS, 100 mM LiCl), and after heating at 65°C with phenol (1:1, 65°C), vortexed for 20 sec and centrifuged at 4°C for 15 min at 12000 rpm. The supernatant was extracted twice with the same volume of phenol:chloroform (1:1) and precipitated with one volume of 4M LiCl. After centrifugation, the RNA pellet was resuspended in TE buffer and two volumes of ethanol were added to the liquid phase to precipitate genomic DNA. Purification of poly(A)$^+$ mRNA was carried out as described [47].

Construction of the yeast two-hybrid cDNA library from wheat cultured cells

Five micrograms of poly(A)$^+$ mRNA isolated from wheat suspension cultured cells were used as a substrate for cDNA synthesis using a cDNA synthesis kit (Stratagene), according to the manufacturer's instructions. The resulting double-stranded DNA, containing EcoRI and XhoI ends, had an average size of 1.3 Kb. A sample (500 ng) of this cDNA was ligated to 750 ng of the EcoRI/XhoI-digested pGAD-GH vector (Clontech) for 48 hr at 8°C. Following ligation, the library was dialyzed against distilled water and electroporated into *E. coli* DH10B (Gibco). For convenience, the cDNA library was separated into five sub-libraries each containing ~$6x10^5$ primary transformants. Total library DNA was obtained by plating primary transformants on fifty 150-mm LB plates plus ampicillin. Colonies were scrapped off into LB (+Amp) medium, and plasmid DNA was prepared as described [34].

Yeast two-hybrid screening

The yeast strain HF7c (*MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1UAS-GAL1TATA-HIS3 URA3::GAL4 17mers(x3)-CyC1TATA-LacZ*; [15]), which contains the two reporter genes *LacZ* and

HIS3, was used in the two-hybrid screening [4, 16]. Yeasts were first transformed, as described [38], with pBWRepA, a plasmid containing the entire WDV RepA open reading frame fused to the Gal4 DNA-binding domain (BD; *TRP1* marker) in the pGBT8 vector [46]. Then, they were transformed with the pGAD-GH (AD; *LEU2* marker) wheat cDNA library. The transformation mixture was plated on yeast drop-out selection media lacking tryptophan, leucine and histidine and supplemented with 5 mM and 10 mM 3-amino-1,2,4,triazole (3-AT; [5]) to reduce the appearance of false positive growing colonies. Transformants were routinely recovered during a 3 to 8 days period and were checked for growth in the presence of up to 20 mM 3-AT. To corroborate the interaction between the two fusion proteins, β-galactosidase activity was assayed by a replica filter assay as described [7]. Plasmid DNA was recovered from positive colonies by transforming into *E. coli* MH4, since this strain is *leuB*$^-$, and its defect can be complemented by the *LEU2* gene present in the pGAD-GH plasmid. Deletions of GRAB1 were constructed using the ApaI (1-253), SalI (1-208), SacI (1-52) and SacII (80-287) restriction sites and deletions of GRAB2 using the XhoI (1-149), BglII (1-108), SalI (1-55) and SmaI (66-351) restriction sites.

Production of GST-fusion proteins and in vitro binding experiments

To produce the GST-GRAB fusion proteins, the oligonucleotide GRAB1-ATG (5'GGATCCATGGTGATGGCAGCGG) and T7 primer, and the oligonucleotides GRAB2-ATG (5'GGATCCATGGCGGACGTGACGGCGGTG) and T7 primer, were used to amplify the coding regions of GRAB1 and GRAB2, respectively by PCR. The products were then cloned in frame into the pGEX-KG vector. The GST-RepA was produced by cloning the WDV RepA ORF in frame into the pGEX-KG vector. *E. coli* BL21(DE3) transformants were grown to an OD_{600} of 0.6 to 0.9 and then induced to express the fusion protein at 37 °C for 30 min by the addition of IPTG to 1 mM. GST fusion proteins were purified using glutathione-Sepharose beads (Pharmacia). Labeled

RepA protein was obtained by *in vitro* transcription and translation (IVT) using wheat germ extract (Promega), in the presence of ^{35}S-methionine, according to the manufacturer's conditions. Labeled GRAB1 and GRAB2 were produced by using TNT reticulocyte lysate (Promega) after cloning the same PCR products from GRAB1 and GRAB2 genes in plasmid pBluescriptKS and transcription using T7 RNA polymerase.

Plant cell culture

The *Triticum monococcum* suspension culture was obtained from P. Mullineaux (John Innes Center, UK) and maintained as described [46].

Inoculation of *N. benthamiana* plants

The PVX-derived pP2C2S vector [10] was used for transient expression of GRAB proteins in *N. benthamiana* plants. For GRAB1 constructions, a 1.1 Kb SmaI-XhoI fragment containing the complete GRAB1 cDNA was cloned into NruI/SalI digested pP2C2S vector to produce plasmid pP2-GRAB1. To construct a frame-shift GRAB1 mutant (GRAB1^{Fs}), plasmid pP2-GRAB1 was partially digested with SacII and, then, religated after treatment with T4 DNA polymerase. For GRAB2 constructions, a 1.35 Kb SmaI-XhoI fragment containing the complete GRAB2 cDNA was cloned into NruI/SalI digested pP2C2S vector to produce plasmid pP2-GRAB2. To construct the frame-shift mutation (GRAB2^{Fs}), plasmid pP2-GRAB2 was digested with BstEII and religated after treatment with Klenow. Infectious RNA was obtained by *in vitro* transcription of plasmid DNA digested with SpeI, using the T7 Cap Scribe kit (Boeringher Mannheim). RNA transcripts were diluted in 5 mM Na_3PO_4 (pH 7.0) and used to inoculate 3-week-old *N. benthamiana* plants (four in each case) using carborundum, as described [10, 17].

Transfection of wheat cultured cells by particle bombardment

Cells were pelleted by centrifugation at 1000 rpm for 3 minutes and the supernatant was removed. Approximately 0.20-0.25 ml of packed cells were spread with a spatula onto a Whatman #1 filter paper, which was placed on CHS medium supplemented with 0.25 M mannitol [30] and solidified with 0.8% agar (bombardment medium). Conditions for DNA adsorption and particle bombardment were as described [43, 46]. Overexpression of GRAB proteins in wheat cultured cells was carried out by cloning the coding regions in a plasmid [47] under the control of the CaMV 35S promoter. The 1.1 Kb EcoRI-XhoI fragment of GRAB1 and the 1.3 Kb EcoRI-ApaII fragment of GRAB2 were cloned into EcoRI/NdeI digested plasmid p35S.ZmRb1 [47] to produce p35S.GRAB1 and p35S.GRAB2. These plasmids contain the 3'-untranslated region of ZmRb1. Each experimental time point corresponds to a cell plate independently transfected. Experiments were repeated at least twice.

Analysis of WDV DNA replication

WDV DNA replication was analyzed essentially as described [43, 46]. Cells were ground in liquid nitrogen and DNA was isolated essentially as described [41] (Soni et al., 1994). After electrophoresis in 0.7% agarose gels, DNA was transferred to nylon membranes (Biodyne A) and detected by hybridization to probes labeled with digoxigenin-11-dUTP according to the conditions recommended by the manufacturer (DIG DNA labeling and detection kit, Boehringer Mannheim).

EXAMPLE 1

Isolation of cDNAs encoding GRAB proteins

Making use of the yeast two-hybrid approach (Fields and Song, 1989; Fields, 1993) a cDNA library was constructed from mRNA prepared from an actively growing wheat cell suspension culture. Screening was carried out using WDV RepA fused to the Gal4 DNA-binding domain. A significantly large number of cDNA clones allowed growth of co-tansformants in selective (-his, +3AT) medium. Among those appeared during the first 6 days after transformation, those co-transformants showing a stronger

interaction, based on their ability to grow in the presence of ≥20 mM 3AT, and to produce an intense β-gal signal. Partial DNA sequence analysis revealed the existence of a group of 7 cDNA clones whose 5'-sequence was significantly related although they represented different clones as deduced by restriction analysis. Based on their ability to interact with WDV RepA,) the proteins encoded by this group of cDNA clones were named GRAB proteins (Geminivirus RepA Binding). Two GRAB proteins, GRAB1 and GRAB2, are described herein.

Each cloned cDNA encoded protein which bound strongly to WDV RepA in yeasts .GRAB-1 and GRAB-2 cDNA clones were ~1.1 kbp long and each contained a single open reading frame, including a putative ATG translation initiation site. The complete cDNA sequence and deduced amino acid sequence for the two GRAB proteins are shown in the sequence listing as SEQ ID Nos 9 to 12. The isolated clones contain the full-length coding region with the sequence around the first putative methionine showing a good consensus translation initiation sequence.

Amino acid analysis of GRAB1 and GRAB2 proteins revealed some striking features. First, the two proteins are totally unrelated in their C-terminal moieties although they appear to be highly related over a region spanning their -170 N-terminal residues, where a significant degree of homology (58%) can be detected. Interestingly, the distribution of charged residues is not random. The unique C-terminal domain of GRAB1 and GRAB2 contains 19% and 15%, respectively, of negatively charged residues (D, E) while their related N-terminal domain, which contains a high proportion of charged. residues (30% and 33%, respectively), show a small bias in favour of positively charged amino acids (R, K, H; 18% and 20%, respectively

In addition, northern analysis revealed the existence of mRNAs of the expected sizes each with the potential to encode GRAB1 and GRAB2, respectively. Both mRNAs were present in small amounts in wheat cultured cells and were even less abundant in differentiated cell types, i. e., roots and leaves .

Example 2.

N-terminus of GRAB proteins mediates binding to WDV RepA

To identify the region in the GRAB proteins involved in complex formation with WDV RepA, a series of deletions were constructed and analyzed for their ability to interact with the viral RepA protein in yeasts. Deletion of most (in GRAB1) or all (in GRAB2) the C-terminal domain did not reduce GRAB-RepA binding (Fig. 2). Even a truncated GRAB2 protein containing only its N-terminal 149 residues still retained a significant RepA binding ability (Fig. 2). On the contrary, a relatively small N-terminal deletion of GRAB1 (80 amino acids) or of GRAB2 (66 amino acids) totally abolished interaction (Fig. 2). Therefore, it is concluded that the N-terminal domain present in both proteins confers the capacity to form complexes with WDV RepA. Furthermore, the most N-terminal region of GRAB proteins appears to have the largest contribution to complex formation with WDV RepA.

Example 3.

C-terminal domain of WDV RepA mediates interaction with GRAB proteins

A similar deletion study was carried out to identify the sequences in the WDV RebA protein responsible for binding to GRAB proteins. As shown in Fig. 3, deletion of most of the N-terminal half of RepA (~ 150 residues) did not decrease its ability to

interact with GRAB proteins. However, elimination of just the C-terminal 37 amino acid residues of RepA completely destroyed binding to both GRAB1 and GRAB2 (Fig. 3), indicating that this small domain of RepA contains residues critical for binding. Interaction of GRAB with WDV Rep protein was also analysed, the other WDV early protein which is produced from the same mRNA encoding RepA but after a splicing event (Schalk et al., 1989). Thus, the 210 N-terminal residues of both RepA and Rep are identical, but the two viral proteins have distinct C-terminal domains. In agreement with the idea that the C-terminus of WDV RepA mediates binding to GRAB, WDV Rep was unable to form complexes with GRAB. These results together with data on the differential binding of WDV RepA and Rep to ZmRbl (Xie et al., 1997) strongly suggest that RepA is a unique WDV protein likely involved in interfering with cellular physiology to create a cellular environment favorable to viral replication.

To confirm and extend the yeast two-hybrid interaction results, pull-down experiments were carrried out to evaluate the interaction using purified proteins. After incubation of equal amounts of purified GST-RepA (0.2μg) with *in vitro* translated (IVT) GST-GRAB1 or GST-GRAB2, a fraction of the input ^{35}S-labeled GRAB proteins was recovered bound to gluthation-agarose beads (Fig. 4). Similar results were obtained using GST-GRAB1 and GST-GRAB2 and IVT WDV RepA protein (Fig. 4). Therefore, it was concluded that interaction between GRAB proteins and the geminiviral RepA can occur in the absence of other cellular proteins.

Example 4

Expression of GRAB mRNAs is restricted to a small number of cells in roots and embryos

To obtain some insight on the function that GRAB proteins may have in the cell, their expression pattern was analyzed by *in situ* hybridization. Northern analysis indicated that GRAB transcripts are not very abundant (see Fig. 1). The occurrence of GRAB mRNAs in root meristems appears to be restricted to a small number of cells A similar patchy pattern was also observed of the histone H4 transcript, characteristic of S-phase cells. In particular, GRAB1 expression was restricted to some cells within the central cylinder and was virtually absent from cortical or epidermal cells. GRAB1 mRNA was also detected in some root cap initial cells . A comparable situation was found in developing embryos

Altogether our analysis of the GRAB expression pattern under different growth conditions led us to conclude that both GRAB1 and GRAB2 mRNA levels increased as a response to changes in growth signals of, perhaps, a subset of cells within the culture and that they are largely dependent on nutrient availability. Furthermore, they reinforce the idea that GRAB proteins may serve different roles as part of an immediate early response, which may be a part of the transduction pathway connecting external signals to the regulation of cellular growth and/or differentiation.

A group of plant proteins is thus identified on the basis of their ability to form complexes with the RepA, the Rb-binding protein of WDV, a member of the plant geminiviridae family. Based on a database searching, we conclude that both GRAB1 and GRAB2 are not homologs to any known protein and, therefore, the cDNAs isolated encode previously unidentified proteins. However, this study revealed that they are related, in terms of primary sequence, throughout their N-terminal region. Using the amino acid sequence of GRAB1 or GRAB2, the output showed that these proteins possess a significant homology to several plant proteins of unknown function.

Interestingly, the homology was also restricted to the N-terminal first 150-170 residues, as initially observed for the group of GRAB proteins itself (Fig. 10A). Those shown in Fig. 10A correspond to otherwise apparently unrelated proteins. First, two Arabidopsis cDNA clones, ATAF1 and ATAF2, isolated by their ability to activate the 35S cauliflower mosaic virus (CAMV) promoter in yeasts (H. Hirt, personal communication). Second, the SENU5 CDNA, isolated in studies of leaf senescence in tomato (Genbank Acc. No.). Third, the NAM protein, the product of the Petunia *No Apical Meristem* (nam) gene, required for proper development of shoot apical meristems, which has been proposed to determine meristem location (Souer et al., 1996).

Example 5

Expression of GRAB 1 induces a necrotic phenotype

As a first step towards getting insight into the cellular roles of GRAB proteins we determined the effect of expressing either GRAB1 or GRAB2 in *N. benthamiana* plants. For this purpose, we made use of a potato virus X (PVX)-based expression vector, which ensures high levels of systemic expression at a given time and in the absence of chromosomal effects [6]. This system has been successfully used to analyze the effects of transiently expressed foreign proteins [18, 31, 32].

When *N. benthamiana* plants were inoculated with *in vitro* transcribed PVX RNA, the appearance of typical symptoms, clearly apparent at 10 days post inoculation (dpi), was indicative of efficient amplification of the PVX expression vector as compared with the mock-inoculated plants Plants inoculated with the PVX-GRAB1 construct were already systemically infected by 12 dpi due to high level amplification of the GRAB1-expressing vector. This is confirmed by the level of PVX-GRAB1 RNA in the leaves, comparable to that of the wild type PVX-infected plants Interestingly, all plants

expressing high levels of GRAB1 showed a tendency to develop, already at 12 dpi, a degenerative process, as revealed by the morphology of their older leaves. Furthermore, a prominent necrotic area appeared near the base of the aerial parts of the plant, especially at 28 dpi . At this stage, a significant reduction in the development of leaves and roots was also apparent. To determine whether the effects observed in whole plants were dependent on the expression of a full-length GRAB1 protein, we inoculated plants with a PVX construct that expressed GRAB1 mRNA carrying a frame-shift mutation close to the N-terminus. Thus, PVX-GRAB1^{Fs} bears a cDNA insert with a frame-shift mutation at amino acid position 78, which maintains the two most N-terminal conserved blocks (N1 and N2), and can produce a truncated protein of 159 residues. Expression of GRAB1^{Fs} did not produce any of the effects observed in plants expressing the full-length GRAB1 protein.

A similar study was carried out with the GRAB2 constructs. Plants infected with the PVX-GRAB2 construct showed delayed kinetics in the PVX vector amplification. This precluded high levels of GRAB2 expression at 12 dpi and plants had a morphology similar to that mock-inoculated plants. However, later after inoculation, the PVX vector accumulated at high levels. Interestingly, these GRAB2-expressing plants showed milder symptoms than plants infected with wild type PVX. None of them developed the degenerative process observed in GRAB1-expressing plants. We also tested the effect of expressing a truncated form of GRAB2. In this case, PVX-GRAB2^{Fs} produces a GRAB2 cDNA carrying a frame-shift mutation at amino acid position 33, thus producing a 50 amino acid-long truncated GRAB2 protein which conserved only the most N-terminal (N1) homology block. Plants inoculated with the PVX-GRAB2^{Fs} construct contain high levels of PVX and of GRAB^{Fs} RNAs. Taken together, the results of expressing the truncated forms of GRAB proteins, indicate that the induction of necrotic areas by GRAB1 and the delay in symptom appearance by GRAB2 are dependent upon

the expression of full-length proteins and strongly suggest that these specific effects may be mediated by the unique C-terminal domains of each GRAB1 and GRAB2 proteins.

The alignment shown in Fig. 4 revealed the existence of several amino acid motifs highly conserved among these related proteins. Thus, we noted the occurrence of five motifs in the N-terminal domain (N1 to N5) which could correspond to blocks critical for their activity. Among them, the two most N-terminal motifs (N1 and N2) exhibit a net negative charge while the rest are positively charged. Based on our deletion analysis, all these motifs are required for efficient interaction with WDV RepA although N5 is not absolutely required and N1 seems to have a strong contribution (Fig. 3). The C-terminal domain, although unique in primary sequence to each protein in the family, shares the property of having a high net negative charge (15-20% of the residues are either D or E). This is particularly evident in both the GRAB proteins and the two ATAF members. The two GRAB proteins reported here, but in particular GRAB2, have a Q-rich domain in their C-terminal domains which could be involved in transcriptional regulation as has been shown to be the case for other examples. In addition, a number of partial cDNA sequences derived from randomly sequenced EST from Arabidopsis and rice were also retrieved using the N-terminus of GRAB proteins as a query (not shown). Surprisingly, protein sequences from yeast or animal origins were not retrieved in this search.

One striking feature of this group of proteins is the large number of members with a related N-terminal domain that appears to be present in each species. For example, at least 5 members related to NAM (Souer et al., 1996) and 7 members related to GRAB (this work). Such an abundance poses the question of whether they actually have different functions. One possibility, already proposed for some NAM-related proteins is

that thay have redundant functions in different locations of the plant during postembryonic development (Souer et al., 1996).

Regarding the consequences of GRAB overexpression on symptom appearance in PVX-infected plants, it is possible that both WDV and PVX share a, so far, unknown pathway affected by GRAB, although very different replication strategies are employed by these virus families. An alternative possibility is that GRAB overexpression may directly or indirectly trigger a general defense pathway or, simply, lead to a cellular environment which protect cells against different types of infection.

Example 6.

Overexpression of GRAB proteins in wheat cultured cells inhibits WDV DNA replication

To further investigate the possible function of the GRAB proteins isolated on the basis of their interaction with WDV RepA protein, we determined the effect of expressing GRAB proteins on geminiviral DNA replication. This assay has proven to be useful to evaluate the effect of plant Rb (ZmRb1) in viral DNA replication [47]. Thus, using a similar strategy, we co-transfected wheat cultured cells with combinations of the following plasmids: (i) one plasmid expressing either GRAB1 or GRAB2 under the control of the 35S CaMV promoter, which is active in the wheat cells used [47], (ii) a second plasmid expressing the WDV proteins required for efficient viral DNA replication (RepA and Rep) also under the control of the 35S CaMV promoter, and (iii) a third plasmid (pWoriΔΔ), a derivative of pWori [43, 46], used to monitor WDV DNA replication, which can replicate efficiently when the viral proteins are provided *in trans* [35, 47]. Expression of either GRAB1 or GRAB2 severely inhibited WDV DNA replication in cultured wheat cells, with GRAB2 exhibiting a stronger effect. These results indicate that WDV DNA replication is affected by GRAB proteins under cell culture conditions.

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(ii) TITLE OF INVENTION: PLANT GRAB PROTEINS

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: ES 9701292
(B) FILING DATE: 12-JUN-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..459

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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420

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(A) LENGTH: 153 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..459

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3;

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1 5 10 15

Xaa Tyr Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Trp Xaa Leu Pro Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Trp Tyr Phe Phe Xaa Xaa Xaa Xaa
50 55 60

Xaa Lys Tyr Pro Xaa Gly Xaa Arg Xaa Asn Arg Xaa Xaa Xaa Xaa Gly
65 70 75 80

Tyr Trp Lys Ala Thr Gly Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Gly Xaa Lys Lys Xaa Leu Val Phe Tyr Xaa Gly Xaa Xaa Xaa
100 105 110

Xaa Gly Xaa Xaa Xaa Xaa Trp Xaa Met His Glu Tyr Arg Leu Xaa Xaa
115 120 125

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
130 135 140

Trp Xaa Xaa Xaa Arg Xaa Xaa Xaa Lys
145 150

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Leu Pro Xaa Gly Phe Arg Phe His Pro Thr Asp Glu Glu Xaa Xaa Xaa
1 5 10 15

Xaa Tyr Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Trp Xaa Leu Pro Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Trp Phe Phe Phe Xaa Xaa Xaa Xaa
50 55 60

Xaa Lys Tyr Pro Xaa Gly Xaa Arg Xaa Asn Arg Xaa Xaa Xaa Xaa Gly
65 70 75 80

Tyr Trp Lys Ala Thr Gly Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Lys Lys Xaa Leu Val Phe Tyr Xaa
100 105 110

Gly Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa Xaa Trp Xaa Met His Glu Tyr
115 120 125

Arg Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
130 135 140

Xaa Trp Xaa Xaa Xaa Arg Xaa Xaa Xaa Lys
145 150

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 459 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..459

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTG CCG CCG GGG TTC CGG TTC CAC CCG ACG GAC GAG GAG CTG GTG GCG
48
Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Ala
1 5 10 15

GAC TAC CTC TGC GCG CGC GCG GCC GGC CGC GCG CCG CCG GTG CCC ATC
96
Asp Tyr Leu Cys Ala Arg Ala Ala Gly Arg Ala Pro Pro Val Pro Ile
20 25 30

ATC GCC GAG CTC GAC CTC TAC CGG TTC GAC CCG TGG GAG CTC CCG GAG
144
Ile Ala Glu Leu Asp Leu Tyr Arg Phe Asp Pro Trp Glu Leu Pro Glu
35 40 45

```
CGG GCG CTC TTC GGG GCG CGG GAG TGG TAC TTC TTC ACG CCG CGG GAC
192
Arg Ala Leu Phe Gly Ala Arg Glu Trp Tyr Phe Phe Thr Pro Arg Asp
     50                  55                  60

CGC AAG TAC CCC AAC GGC TCC CGC CCC AAC CGG GCC GCC GGG GGC GGC
240
Arg Lys Tyr Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Gly Gly
 65                  70                  75                  80

TAC TGG AAG GCC ACC GGC GCC GAC AGG CCC GTG GCG CGC GCG GGC AGG
288
Tyr Trp Lys Ala Thr Gly Ala Asp Arg Pro Val Ala Arg Ala Gly Arg
                 85                  90                  95

ACC GTC GGG ATC AAG AAG GCG CTC GTC TTC TAC CAC GGC AGG CCG TCG
336
Thr Val Gly Ile Lys Lys Ala Leu Val Phe Tyr His Gly Arg Pro Ser
            100                 105                 110

GCG GGG GTC AAG ACG GAC TGG ATC ATG CAC GAG TAC CGC CTC GCC GGC
384
Ala Gly Val Lys Thr Asp Trp Ile Met His Glu Tyr Arg Leu Ala Gly
        115                 120                 125

GCC GAC GGA CGC GCC GCC AAG AAC GGC GGC ACG CTC AGG CTT GAC GAA
432
Ala Asp Gly Arg Ala Ala Lys Asn Gly Gly Thr Leu Arg Leu Asp Glu
    130                 135                 140

TGG GTG CTC TGC CGC CTA TAC AAC AAG
459
Trp Val Leu Cys Arg Leu Tyr Asn Lys
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Ala
  1               5                  10                  15

Asp Tyr Leu Cys Ala Arg Ala Ala Gly Arg Ala Pro Pro Val Pro Ile
             20                  25                  30

Ile Ala Glu Leu Asp Leu Tyr Arg Phe Asp Pro Trp Glu Leu Pro Glu
         35                  40                  45

Arg Ala Leu Phe Gly Ala Arg Glu Trp Tyr Phe Phe Thr Pro Arg Asp
     50                  55                  60
```

```
Arg Lys Tyr Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Gly Gly
 65                  70                  75                  80

Tyr Trp Lys Ala Thr Gly Ala Asp Arg Pro Val Ala Arg Ala Gly Arg
                 85                  90                  95

Thr Val Gly Ile Lys Lys Ala Leu Val Phe Tyr His Gly Arg Pro Ser
            100                 105                 110

Ala Gly Val Lys Thr Asp Trp Ile Met His Glu Tyr Arg Leu Ala Gly
        115                 120                 125

Ala Asp Gly Arg Ala Ala Lys Asn Gly Gly Thr Leu Arg Leu Asp Glu
    130                 135                 140

Trp Val Leu Cys Arg Leu Tyr Asn Lys
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..462

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
CTT CCA CCG GGG TTC CGG TTC CAC CCC ACC GAC GAG GAG GTG GTC ACC
48
Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Val Val Thr
    155                 160                 165

CAC TAC CTC ACC CGC AAG GTC CTC CGC GAA TCC TTC TCC TGC CAA GTG
96
His Tyr Leu Thr Arg Lys Val Leu Arg Glu Ser Phe Ser Cys Gln Val
170                 175                 180                 185

ATC ACC GAC GTC GAC CTC AAC AAG AAC GAG CCG TGG GAG CTC CCG GGC
144
Ile Thr Asp Val Asp Leu Asn Lys Asn Glu Pro Trp Glu Leu Pro Gly
                190                 195                 200
```

```
CTC GCG AAG ATG GGC GAG AAG GAG TGG TTC TTC TTC GCG CAC AAG GGT
192
Leu Ala Lys Met Gly Glu Lys Glu Trp Phe Phe Phe Ala His Lys Gly
            205                 210                 215

CGG AAG TAC CCG ACG GGG ACG CGC ACC AAC CGG GCG ACG AAG AAG GGG
240
Arg Lys Tyr Pro Thr Gly Thr Arg Thr Asn Arg Ala Thr Lys Lys Gly
        220                 225                 230

TAC TGG AAG GCG ACG GGG AAG GAC AAG GAG ATC TTC CGC GGC AAG GGC
288
Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg Gly Lys Gly
    235                 240                 245

CGG GAC GCC GTC CTT GTC GGC ATG AAG AAG ACG CTC GTC TTT TAC ACC
336
Arg Asp Ala Val Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr
250                 255                 260                 265

GGC CGC GCC CCC AGC GGC GGG AAG ACG CCG TGG GTG ATG CAC GAG TAC
384
Gly Arg Ala Pro Ser Gly Gly Lys Thr Pro Trp Val Met His Glu Tyr
                270                 275                 280

CGC CTC GAG GGC GAG CTG CCC CAT CGC CTT CCC CGC ACC GCC AAG GAC
432
Arg Leu Glu Gly Glu Leu Pro His Arg Leu Pro Arg Thr Ala Lys Asp
            285                 290                 295

GAT TGG GCT GTT TGC CGG GTG TTC AAC AAA
462
Asp Trp Ala Val Cys Arg Val Phe Asn Lys
        300                 305
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Val Val Thr
 1               5                  10                  15

His Tyr Leu Thr Arg Lys Val Leu Arg Glu Ser Phe Ser Cys Gln Val
            20                  25                  30

Ile Thr Asp Val Asp Leu Asn Lys Asn Glu Pro Trp Glu Leu Pro Gly
        35                  40                  45

Leu Ala Lys Met Gly Glu Lys Glu Trp Phe Phe Phe Ala His Lys Gly
    50                  55                  60
```

```
Arg Lys Tyr Pro Thr Gly Thr Arg Thr Asn Arg Ala Thr Lys Lys Gly
 65                  70                  75                  80

Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg Gly Lys Gly
                85                  90                  95

Arg Asp Ala Val Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr
            100                 105                 110

Gly Arg Ala Pro Ser Gly Gly Lys Thr Pro Trp Val Met His Glu Tyr
        115                 120                 125

Arg Leu Glu Gly Glu Leu Pro His Arg Leu Pro Arg Thr Ala Lys Asp
    130                 135                 140

Asp Trp Ala Val Cys Arg Val Phe Asn Lys
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1090 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:94..954

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
AATTCGGCAC GAGACAGTCC ACCACGCACG TGCAGCAGCA CCAGCGCCCG AGAATCCCAT
60

TCCCATCGAC GGAGAAGAAG AAGTGAAGAA ACA ATG GTG ATG GCA GCG GCG GAG
114
                                     Met Val Met Ala Ala Ala Glu
                                     155                 160

CGG CGG GAC GCG GAG GCG GAG CTG AAC CTG CCG CCG GGG TTC CGG TTC
162
Arg Arg Asp Ala Glu Ala Glu Leu Asn Leu Pro Pro Gly Phe Arg Phe
            165                 170                 175

CAC CCG ACG GAC GAG GAG CTG GTG GCG GAC TAC CTC TGC GCG CGC GCG
210
His Pro Thr Asp Glu Glu Leu Val Ala Asp Tyr Leu Cys Ala Arg Ala
```

```
          180                 185                 190

GCC GGC CGC GCG CCG CCG GTG CCC ATC ATC GCC GAG CTC GAC CTC TAC
258
Ala Gly Arg Ala Pro Pro Val Pro Ile Ile Ala Glu Leu Asp Leu Tyr
195                 200                 205

CGG TTC GAC CCG TGG GAG CTC CCG GAG CGG GCG CTC TTC GGG GCG CGG
306
Arg Phe Asp Pro Trp Glu Leu Pro Glu Arg Ala Leu Phe Gly Ala Arg
210                 215                 220                 225

GAG TGG TAC TTC TTC ACG CCG CGG GAC CGC AAG TAC CCC AAC GGC TCC
354
Glu Trp Tyr Phe Phe Thr Pro Arg Asp Arg Lys Tyr Pro Asn Gly Ser
                230                 235                 240

CGC CCC AAC CGG GCC GCC GGG GGC GGC TAC TGG AAG GCC ACC GGC GCC
402
Arg Pro Asn Arg Ala Ala Gly Gly Gly Tyr Trp Lys Ala Thr Gly Ala
            245                 250                 255

GAC AGG CCC GTG GCG CGC GCG GGC AGG ACC GTC GGG ATC AAG AAG GCG
450
Asp Arg Pro Val Ala Arg Ala Gly Arg Thr Val Gly Ile Lys Lys Ala
        260                 265                 270

CTC GTC TTC TAC CAC GGC AGG CCG TCG GCG GGG GTC AAG ACG GAC TGG
498
Leu Val Phe Tyr His Gly Arg Pro Ser Ala Gly Val Lys Thr Asp Trp
    275                 280                 285

ATC ATG CAC GAG TAC CGC CTC GCC GGC GCC GAC GGA CGC GCC GCC AAG
546
Ile Met His Glu Tyr Arg Leu Ala Gly Ala Asp Gly Arg Ala Ala Lys
290                 295                 300                 305

AAC GGC GGC ACG CTC AGG CTT GAC GAA TGG GTG CTC TGC CGC CTA TAC
594
Asn Gly Gly Thr Leu Arg Leu Asp Glu Trp Val Leu Cys Arg Leu Tyr
                310                 315                 320

AAC AAG AAG AAC CAG TGG GAG AAG ATG CAG CGG CAG CGG CAG GAG GAG
642
Asn Lys Lys Asn Gln Trp Glu Lys Met Gln Arg Gln Arg Gln Glu Glu
            325                 330                 335

GAG GCG GCG GCC AAG GCT GCG GCG TCA CAG TCG GTC TCC TGG GGT GAG
690
Glu Ala Ala Ala Lys Ala Ala Ala Ser Gln Ser Val Ser Trp Gly Glu
        340                 345                 350

ACG CGG ACG CCG GAG TCC GAC GTC GAC AAC GAT CCG TTC CCG GAG CTG
738
Thr Arg Thr Pro Glu Ser Asp Val Asp Asn Asp Pro Phe Pro Glu Leu
    355                 360                 365
```

```
GAC TCG CTG CCG GAG TTC CAG ACG GCA AAC GCG TCA ATA CTG CCC AAG
786
Asp Ser Leu Pro Glu Phe Gln Thr Ala Asn Ala Ser Ile Leu Pro Lys
370                 375                 380                 385

GAG GAG GTG CAG GAG CTG GGC AAC GAC GAC TGG CTC ATG GGG ATC AGC
834
Glu Glu Val Gln Glu Leu Gly Asn Asp Asp Trp Leu Met Gly Ile Ser
                390                 395                 400

CTC GAC GAC CTG CAG GGC CCC GGC TCC CTG ATG CTG CCC TGG GAC GAC
882
Leu Asp Asp Leu Gln Gly Pro Gly Ser Leu Met Leu Pro Trp Asp Asp
            405                 410                 415

TCC TAC GCC GCC TCG TTC CTG TCG CCG GTG GCC ACG ATG AAG ATG GAG
930
Ser Tyr Ala Ala Ser Phe Leu Ser Pro Val Ala Thr Met Lys Met Glu
        420                 425                 430

CAG GAC GTC AGC CCA TTC TTC TTC TGAGCTCTCA ATACTCTCAC GGTCGCACTG
984
Gln Asp Val Ser Pro Phe Phe Phe
    435                 440

TTGTGTGCGG CGTAACTGTA GATAGTTCAC ATTTGTTCAG GATTTATTTG TAACGTTGCT
1044

TCTTTTATAC GATACTCTCT TCCTTTCTAA AAAAAAAAAA AAAAAA
1090
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Met Val Met Ala Ala Ala Glu Arg Arg Asp Ala Glu Ala Glu Leu Asn
 1               5                  10                  15

Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Ala
            20                  25                  30

Asp Tyr Leu Cys Ala Arg Ala Ala Gly Arg Ala Pro Pro Val Pro Ile
        35                  40                  45

Ile Ala Glu Leu Asp Leu Tyr Arg Phe Asp Pro Trp Glu Leu Pro Glu
    50                  55                  60

Arg Ala Leu Phe Gly Ala Arg Glu Trp Tyr Phe Phe Thr Pro Arg Asp
65                  70                  75                  80

Arg Lys Tyr Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Gly Gly
```

85 90 95

Tyr Trp Lys Ala Thr Gly Ala Asp Arg Pro Val Ala Arg Ala Gly Arg
100 105 110

Thr Val Gly Ile Lys Lys Ala Leu Val Phe Tyr His Gly Arg Pro Ser
115 120 125

Ala Gly Val Lys Thr Asp Trp Ile Met His Glu Tyr Arg Leu Ala Gly
130 135 140

Ala Asp Gly Arg Ala Ala Lys Asn Gly Gly Thr Leu Arg Leu Asp Glu
145 150 155 160

Trp Val Leu Cys Arg Leu Tyr Asn Lys Lys Asn Gln Trp Glu Lys Met
165 170 175

Gln Arg Gln Arg Gln Glu Glu Glu Ala Ala Ala Lys Ala Ala Ala Ser
180 185 190

Gln Ser Val Ser Trp Gly Glu Thr Arg Thr Pro Glu Ser Asp Val Asp
195 200 205

Asn Asp Pro Phe Pro Glu Leu Asp Ser Leu Pro Glu Phe Gln Thr Ala
210 215 220

Asn Ala Ser Ile Leu Pro Lys Glu Glu Val Gln Glu Leu Gly Asn Asp
225 230 235 240

Asp Trp Leu Met Gly Ile Ser Leu Asp Asp Leu Gln Gly Pro Gly Ser
245 250 255

Leu Met Leu Pro Trp Asp Asp Ser Tyr Ala Ala Ser Phe Leu Ser Pro
260 265 270

Val Ala Thr Met Lys Met Glu Gln Asp Val Ser Pro Phe Phe Phe
275 280 285

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1295 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum monococcum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:109..1161

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
ATTCGGCACG AGATCACCTC TAACATCTCG ATCTACCTCT TCCTCCTCCT CAGCTCTCGT
60

TCCATCAGGT TCTTCCACAG CGTAGCAAGG CAATCTAGTA GATCCTCC ATG TCG GAC
117
                                                        Met Ser Asp
                                                                290

GTG ACG GCG GTG ATG GAT CTG GAG GTG GAG GAG CCG CAG CTG GCG CTT
165
Val Thr Ala Val Met Asp Leu Glu Val Glu Glu Pro Gln Leu Ala Leu
                295                 300                 305

CCA CCG GGG TTC CGG TTC CAC CCC ACC GAC GAG GAG GTG GTC ACC CAC
213
Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Val Val Thr His
            310                 315                 320

TAC CTC ACC CGC AAG GTC CTC CGC GAA TCC TTC TCC TGC CAA GTG ATC
261
Tyr Leu Thr Arg Lys Val Leu Arg Glu Ser Phe Ser Cys Gln Val Ile
        325                 330                 335

ACC GAC GTC GAC CTC AAC AAG AAC GAG CCG TGG GAG CTC CCG GGC CTC
309
Thr Asp Val Asp Leu Asn Lys Asn Glu Pro Trp Glu Leu Pro Gly Leu
    340                 345                 350

GCG AAG ATG GGC GAG AAG GAG TGG TTC TTC TTC GCG CAC AAG GGT CGG
357
Ala Lys Met Gly Glu Lys Glu Trp Phe Phe Phe Ala His Lys Gly Arg
355                 360                 365                 370

AAG TAC CCG ACG GGG ACG CGC ACC AAC CGG GCG ACG AAG AAG GGG TAC
405
Lys Tyr Pro Thr Gly Thr Arg Thr Asn Arg Ala Thr Lys Lys Gly Tyr
                375                 380                 385

TGG AAG GCG ACG GGG AAG GAC AAG GAG ATC TTC CGC GGC AAG GGC CGG
453
Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg Gly Lys Gly Arg
            390                 395                 400

GAC GCC GTC CTT GTC GGC ATG AAG AAG ACG CTC GTC TTT TAC ACC GGC
501
Asp Ala Val Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr Gly
        405                 410                 415

CGC GCC CCC AGC GGC GGG AAG ACG CCG TGG GTG ATG CAC GAG TAC CGC
549
Arg Ala Pro Ser Gly Gly Lys Thr Pro Trp Val Met His Glu Tyr Arg
    420                 425                 430
```

```
CTC GAG GGC GAG CTG CCC CAT CGC CTT CCC CGC ACC GCC AAG GAC GAT
597
Leu Glu Gly Glu Leu Pro His Arg Leu Pro Arg Thr Ala Lys Asp Asp
435                 440                 445                 450

TGG GCT GTT TGC CGG GTG TTC AAC AAA GAC TTG GCG GCG AGG AAT GCG
645
Trp Ala Val Cys Arg Val Phe Asn Lys Asp Leu Ala Ala Arg Asn Ala
                455                 460                 465

CCC CAG ATG GCG CCG GCG GCC GAC GGT GGC ATG GAG GAC CCG CTC GCC
693
Pro Gln Met Ala Pro Ala Ala Asp Gly Gly Met Glu Asp Pro Leu Ala
            470                 475                 480

TTC CTC GAT GAC TTG CTC ATC GAC ACC GAC CTG TTC GAC GAC GCG GAC
741
Phe Leu Asp Asp Leu Leu Ile Asp Thr Asp Leu Phe Asp Asp Ala Asp
        485                 490                 495

CTG CCG ATG CTC ATG GAC TCT CCG TCT GGC GCT GAC GAC TTC GCC GGC
789
Leu Pro Met Leu Met Asp Ser Pro Ser Gly Ala Asp Asp Phe Ala Gly
    500                 505                 510

GCT TCG AGC TCC ACC TGC AGC GCG GCC CTG CCG CTT GAG CCG GAC GCG
837
Ala Ser Ser Ser Thr Cys Ser Ala Ala Leu Pro Leu Glu Pro Asp Ala
515                 520                 525                 530

GAG CTA CCG GTG CTG CAT CCG CAG CAG CAG CAG AGC CCC AAC TAC TTC
885
Glu Leu Pro Val Leu His Pro Gln Gln Gln Gln Ser Pro Asn Tyr Phe
                535                 540                 545

TTC ATG CCG GCG ACG GCC AAC GGC AAT CTT GGC GGC GCC GAG TAC TCA
933
Phe Met Pro Ala Thr Ala Asn Gly Asn Leu Gly Gly Ala Glu Tyr Ser
            550                 555                 560

CCC TAC CAG GCT ATG GGG GAC CAG CAG GCC GCG ATC CGC AGG TAC TGC
981
Pro Tyr Gln Ala Met Gly Asp Gln Gln Ala Ala Ile Arg Arg Tyr Cys
        565                 570                 575

AAG CCG AAG GCG GAG GTA GCG TCT TCG TCG GCG CTG CTG AGC CCT TCG
1029
Lys Pro Lys Ala Glu Val Ala Ser Ser Ser Ala Leu Leu Ser Pro Ser
    580                 585                 590

CTG GGC TTG GAC ACG GCG GCG CTT GCC GGC GCG GAG ACC TCC TTC CTG
1077
Leu Gly Leu Asp Thr Ala Ala Leu Ala Gly Ala Glu Thr Ser Phe Leu
595                 600                 605                 610

ATG CCG TCA TCG CGG TCG TAC CTC GAT CTG GAG GAG CTG TTC CGG GGC
1125
Met Pro Ser Ser Arg Ser Tyr Leu Asp Leu Glu Glu Leu Phe Arg Gly
```

615 620 625

GAG CCT CTC ATG GAC TAC TCC AAC ATG TGG AAG ATC TGATGTGGAA
1171
Glu Pro Leu Met Asp Tyr Ser Asn Met Trp Lys Ile
630 635

GATCTGGAGC GTCTCAGTTT GCTGGTAGCT ATAGATGGGT ATTTGGTTGA TGCTAGCTCT
1231

TCGACTGATT AGTTGCTTCA TTAACTTTCG ATTAAGGATT GAGTTAAAAA AAAAAAAAAA
1291

AAAA
1295

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Met Ser Asp Val Thr Ala Val Met Asp Leu Glu Val Glu Glu Pro Gln
1 5 10 15

Leu Ala Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Val
20 25 30

Val Thr His Tyr Leu Thr Arg Lys Val Leu Arg Glu Ser Phe Ser Cys
35 40 45

Gln Val Ile Thr Asp Val Asp Leu Asn Lys Asn Glu Pro Trp Glu Leu
50 55 60

Pro Gly Leu Ala Lys Met Gly Glu Lys Glu Trp Phe Phe Phe Ala His
65 70 75 80

Lys Gly Arg Lys Tyr Pro Thr Gly Thr Arg Thr Asn Arg Ala Thr Lys
85 90 95

Lys Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg Gly
100 105 110

Lys Gly Arg Asp Ala Val Leu Val Gly Met Lys Lys Thr Leu Val Phe
115 120 125

Tyr Thr Gly Arg Ala Pro Ser Gly Gly Lys Thr Pro Trp Val Met His
130 135 140

Glu Tyr Arg Leu Glu Gly Glu Leu Pro His Arg Leu Pro Arg Thr Ala
145 150 155 160

Lys Asp Asp Trp Ala Val Cys Arg Val Phe Asn Lys Asp Leu Ala Ala

```
                    165                 170                 175

Arg Asn Ala Pro Gln Met Ala Pro Ala Ala Asp Gly Gly Met Glu Asp
            180                 185                 190

Pro Leu Ala Phe Leu Asp Asp Leu Leu Ile Asp Thr Asp Leu Phe Asp
        195                 200                 205

Asp Ala Asp Leu Pro Met Leu Met Asp Ser Pro Ser Gly Ala Asp Asp
    210                 215                 220

Phe Ala Gly Ala Ser Ser Ser Thr Cys Ser Ala Ala Leu Pro Leu Glu
225                 230                 235                 240

Pro Asp Ala Glu Leu Pro Val Leu His Pro Gln Gln Gln Gln Ser Pro
                245                 250                 255

Asn Tyr Phe Phe Met Pro Ala Thr Ala Asn Gly Asn Leu Gly Gly Ala
            260                 265                 270

Glu Tyr Ser Pro Tyr Gln Ala Met Gly Asp Gln Gln Ala Ala Ile Arg
        275                 280                 285

Arg Tyr Cys Lys Pro Lys Ala Glu Val Ala Ser Ser Ser Ala Leu Leu
    290                 295                 300

Ser Pro Ser Leu Gly Leu Asp Thr Ala Ala Leu Ala Gly Ala Glu Thr
305                 310                 315                 320

Ser Phe Leu Met Pro Ser Ser Arg Ser Tyr Leu Asp Leu Glu Glu Leu
                325                 330                 335

Phe Arg Gly Glu Pro Leu Met Asp Tyr Ser Asn Met Trp Lys Ile
            340                 345                 350
```

Applicant's or agent's file reference number 198.091/EXT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 9, line 20

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

COLECCIÓN ESPAÑOLA DE CULTIVOS TIPO

Address of depositary institution *(including postal code and country)*

Microbiology Department
Biological Science Faculty/UNIVERSITY OF VALENCIA
46100 Burjasot /Valencia/Spain

Date of deposit	Accession Number
11th June 1997	CECT 4889

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer : L.R. Pether	Authorized officer

Form PCT/RO/134 (July 1992)

Applicant's or agent's file reference number	198.091/EXT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 9, line 21

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

COLECCIÓN ESPAÑOLA DE CULTIVOS TIPO

Address of depositary institution *(including postal code and country)*

Microbiology Department
Biological Science Faculty/UNIVERSITY OF VALENCIA
46100 Burjasot/Valencia/Spain

Date of deposit	Accession Number
11th June 1997	CECT 4890

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer: L.R. Pether	Authorized officer

CLAIMS.

1. A method of controlling plant cell cycle characterised in that it comprises increasing or decreasing the levels of, or Geminivirus RepA binding capabilities of, GRAB (Geminivirus RepA Binding) proteins or peptides within a plant cell.

2. A method as claimed in claim 1 characterised in that the control of the plant cell cycle comprises one or more of control of plant cell or plant virus growth and/or replication, plant cell differentiation, development and/or scenescence

3. A method as claimed in claim 1 or claim 2 characterised in that the GRAB proteins or peptides comprise domains N1, N2, N3, N4 and N5 as shown in figure 4 herein

4. A method as claimed in any one of the preceding claims wherein the GRAB proteins or peptides have a first 150 N-terminal amino acids capable of binding to viral RepA protein

5. A method as claimed in any one of the preceding claims characterised in that the GRAB proteins or peptide comprises an amino acid sequence SEQ ID No 3 or 4 as shown herein or a functional variant thereof that is capable of binding Geminivirus RepA

6. A method as claimed in any one of the preceding claims characterised in that it comprises overproducing or underproducing the protein or peptide in a plant cell.

7. A method as claimed in any one of claims 1 to 6 characterised in that it comprises decrease of native GRAB binding activity by application of an agent that binds to GRAB protein or peptide.

8. A method as claimed in any one of the preceding claims characterised in that the GRAB proteins or peptides have amino acid sequence homology of at least 70% with that of SEQ ID No 3 or 4.

9. A method as claimed in any one of the preceding claims comprising placing of the corresponding GRAB protein or peptide encoding or antisense nucleotides within the plant cell.

10. A method as claimed in claim 9 characterised in that the nucleotides are in the form of recombinant nucleic acid comprising a GRAB protein or peptide encoding sequence.

11. A method as claimed in claim 10 characterised in that the sequence is positioned behind a promotor capable of supporting GRAB protein or peptide expression or production of antisense RNA.

12. A method as claimed in any one of claims 1 to 11 characterised in that the protein or peptide is applied or produced ectopically.

13. A method as claimed in claim 12 characterised in that the tissue is vegetative tissue or stem tissue.

14. A method as claimed in any one of the preceding claims comprising expressing a protein or peptide that is capable of binding to GRAB protein or peptide or functional variant thereof within the cell.

15. A method as claimed in any preceding claim characterised in that it comprises downregulating native GRAB expression by gene silencing coexpression or through antisense strategy.

16. A method as claimed in any one of the preceding claims characterised in that it comprises producing or inhibiting senescence in a plant cell comprising increasing or decreasing the levels or binding activity of a GRAB protein or peptide comprising a sequence of SEQ ID No 10 or a functional variant therof capable of inducing senescence in *N.bentamiana* plants, in a plant cell.

17. A method as claimed in claim 16 comprising incorporation of nucleic acid encoding RepA, N-terminal truncated RepA or a functional variant of one of these.

18. A GRAB protein or peptide *per se*, or in enriched, isolated, cell free and/or recombinantly produced form with the proviso that it is not one of SENU, NAM, ATAF1 or ATAF2.

19. A protein or peptide as claimed in claim 18 characterised in it has an N-terminal sequence having 90% or more homology to the first 150 N-terminal amino acids of GRAB1 or GRAB2 described herein or conservatively substituted variants thereof.

20. A GRAB protein or peptide as claimed in claim 18 characterised in that it comprises an amino acid sequence SEQ ID No 3 or 4 as shown or a functional variant thereof having an amino acid sequence of homology of at least 70% with that sequence that is capable of binding Geminivirus RepA.

21. A protein or peptide as claimed in claim 20 characterised in that it comprises a sequence of SEQ ID No 6 or 8 or a functional variant thereof having an amino acid sequence of homology of at least 70% with that sequence.

22. A protein or peptide as claimed in claim 21 characterised in that it comprises a sequence of SEQ ID No 10 or 12 or a functional variant thereof having an amino acid sequence of homology of at least 70% with that sequence.

23. A GRAB protein or peptide encoding or antisense nucleic acid *per se* , or in enriched, isolated, cell free and/or recombinant form with the proviso that it does not encode the full amino acid sequence of SENU, NAM, ATAF1 or ATAF2.

24. Nucleic acid as claimed in claim 23 characterised in that it is in the form of recombinant DNA or cRNA (mRNA) that codes for the expression of a GRAB protein or peptide having an N-terminal sequence with at least 60% homology with the first 200 N-terminal amino acids of GRAB1 or GRAB2 as described herein

25. A nucleic acid as claimed in claim 24 characterised in that it is a DNA or RNA polynucleotide comprising one or more of SEQ ID No 1, 2, 5, 7, 9 or 11 or a functional variant thereof

26. A method of producing a protein or peptide as claimed in any one of claims 18 to 23 characterised in that it comprises expressing DNA or RNA as described in claim 24 or 25..

27. A nucleic acid probe or primer characterised in that it comprises an oligonucleotide or polynucleotide of sequence complementary to any 15 or more contiguous bases of the DNA sequences identified herein below as SEQ ID No 5, 7, 9 or 11 or complemetary sequences or RNA sequences corresponding thereto.

28. A nucleic acid transformation vector characterised in that it comprises DNA or RNA as described in any one of claims 9 to 17 or 23 to 25.

29. A method for producing transformed cells comprising nucleic acid as claimed in or described in any one of claims 9 to 17 or 23 to 25 comprising introducing said nucleic acid into the cell in vector or free form.

30. A method as claimed in claim 29 characterised in that the nucleic acid is introduced directly by electroporation or particle bombardment.

31. A cell comprising recombinant nucleic acid as described or claimed in any one of claims 9 to 17 or 23 to 25.

32. A transgenic plant or part thereof comprising a cell as claimed in claim 31.

33. A plasmid containing a DNA coding for expression of GRAB protein GRAB 1 or GRAB 2 described herein as deposited under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms of 1977; these being deposited on 11 June 1997 at the Coleccion Espanola de Cultivos Tipo, with the accession numbers CECT 4889 or CECT 4890 .

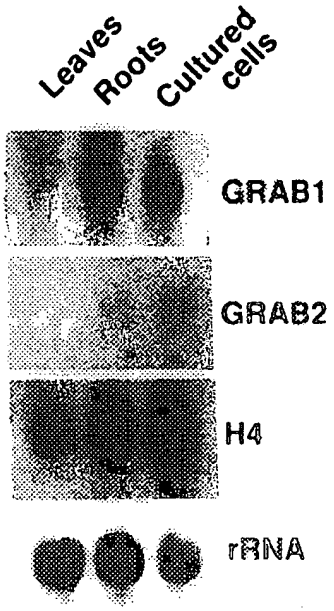


FIG 1

FIG 2

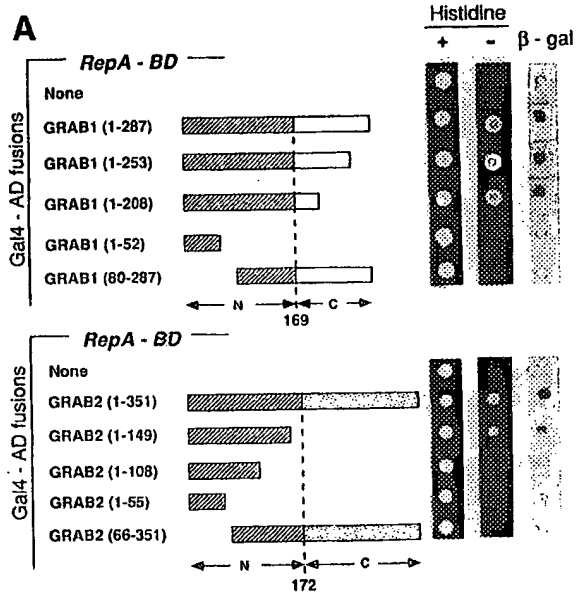
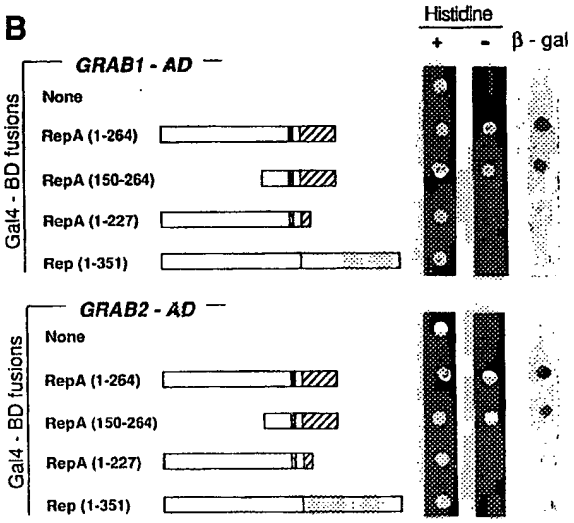
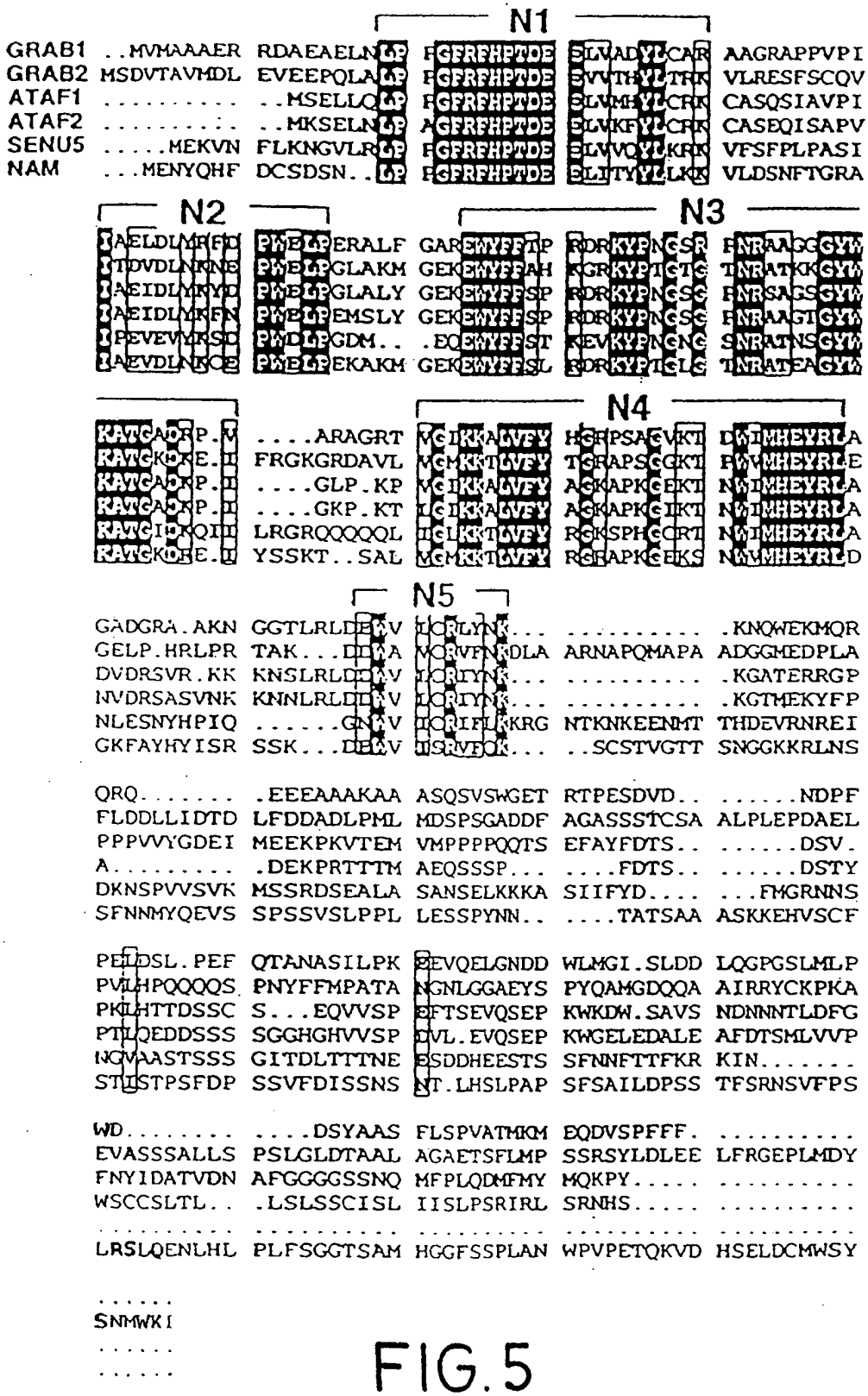

A
RepA - BD
Gal4 - AD fusions
None
GRAB1 (1-287)
GRAB1 (1-253)
GRAB1 (1-208)
GRAB1 (1-52)
GRAB1 (60-287)
N
C
169
RepA - BD
Gal4 - AD fusions
None
GRAB2 (1-351)
GRAB2 (1-149)
GRAB2 (1-108)
GRAB2 (1-55)
GRAB2 (66-351)
N
C
172
Histidine
+
-
β - gal

FIG 3


B
Histidine
+
-
β - gal
GRAB1 - AD
Gal4 - BD fusions
None
RepA (1-264)
RepA (150-264)
RepA (1-227)
Rep (1-351)
GRAB2 - AD
Gal4 - BD fusions
None
RepA (1-264)
RepA (150-264)
RepA (1-227)
Rep (1-351)

	Day 2						Day 3					
p35S.A+B	+	+	+	+	+	+	+	+	+	+	+	+
p35S.GRAB1		+	+					+	+			
p35S.GRAB2					+	+					+	+
GRAB/A+B ratio		2	6		2	6		2	6		2	6

FIG 4


N1
N2
N3
N4
N5
GRAB1 ..MVMAAAAER RDAEAELN
GRAB2 MSDVTAVMDL EVEEPQLA
ATAF1 ....MSELLQ
ATAF2 ....MKSELN
SENU5 .....MEKVN FLKNGVLR
NAM ...MENYQHF DCSDSN..
FIG.5

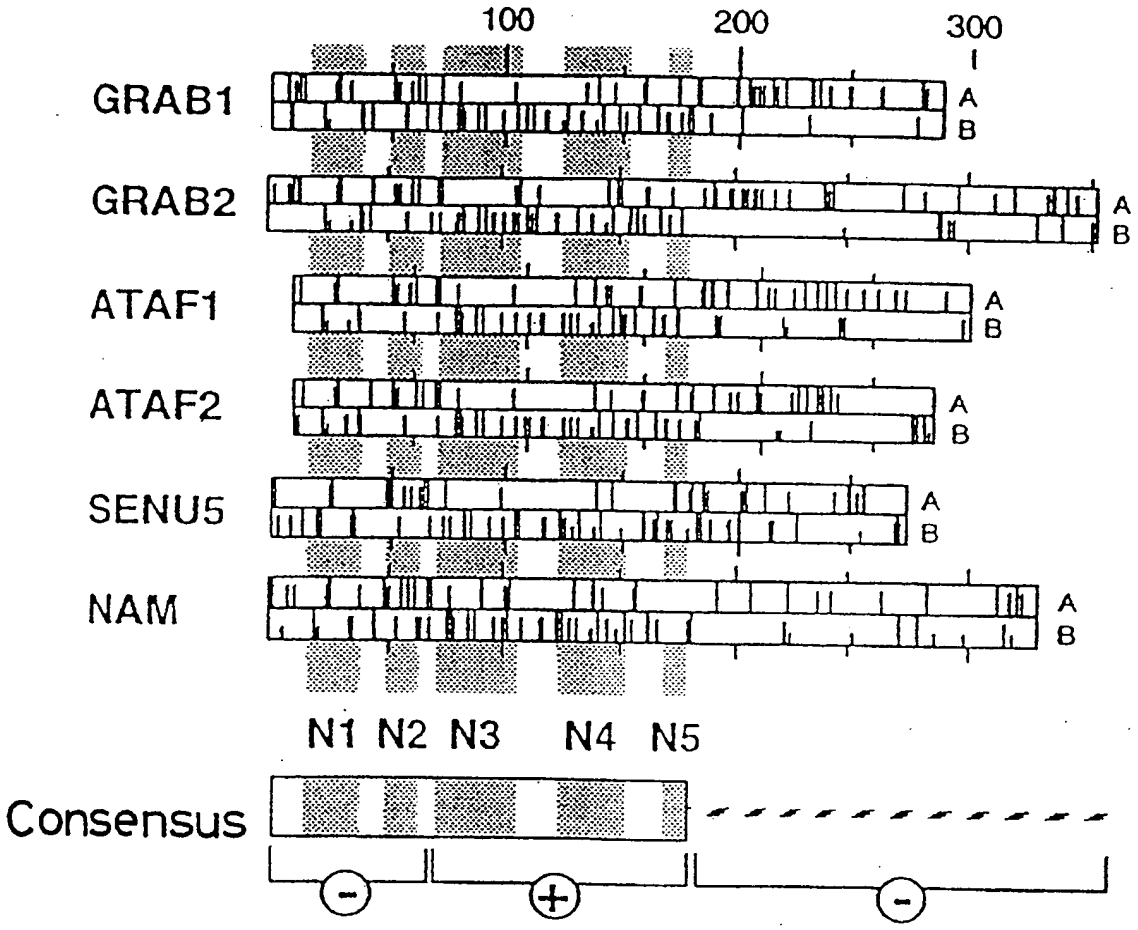

100
200
300
GRAB1
GRAB2
ATAF1
ATAF2
SENU5
NAM
A
B
N1 N2 N3 N4 N5
Consensus
-
+
-

FIG. 6